REMARKS

Claim Rejections Under 35 U.S.C. §101 and § 112, first paragraph

Claims 39-43 stand rejected under 35 U.S.C. §101 as allegedly lacking a specific, substantial and credible asserted utility or a well established utility. In the Examiner's Answer, the Examiner cites the following arguments and cites new references in support of these conclusions.

(1) The genomic DNA encoding PRO269 had a ΔCt value of at least 1.0 for eight out of seventeen lung tumor samples. Genomic DNA encoding PRO269 was not amplified in any of the seventeen colon tumor samples (Examiner's Answer, page 4 –5).

(2) In order for PRO269 polypeptides to be overexpressed in lung tumors, amplified genomic DNA would have to correlate with amplified mRNA. The art allegedly discloses that such correlations cannot be presumed. (Examiner's Answer page 5). The Examiner cites Pennica *et al.*, and Konopka *et al.*

(3) In order for PRO269 polypeptides to be overexpressed in lung tumors, amplified mRNA would have to correlate with amplified polypeptide. (Examiner's Answer, page 5). The art allegedly discloses that such correlations cannot be presumed. The Examiner cites Hu et al (2003), Haynes et al., (1999) and **new references** LaBaer (2003), Gygi *et al.* (1999), Chen *et al.* (2002), Lian *et al.* (2001), Fessler *et al.* (2002) and Greenbaum (2003) for support.

The same arguments are cited in support of the rejection of claims 39-43 under 35 U.S.C. §112, first paragraph, for alleged lack of enablement for how to use the invention, since the claimed invention is allegedly not supported by either a credible, specific and substantial asserted utility or a well established utility.

Applicants disagree with each of the Examiner's arguments on a number of grounds.

The Examiner's arguments will be addressed in the order they are listed above.

(1) In making the rejection that "only eight out of seventeen lung tumor samples tested positive" and "PRO269 was not amplified in any of the seventeen **colon** tumor samples" (emphasis added) (Examiner's Answer, pages 4-5), the Examiner seems to indicate that a tumor marker is patentable only if the marker tests positive in a statistically high number of samples compared to

the total number of samples tested or if the tumor tests positive in every tissue type that was studied.

Applicants have presented arguments why this rejection is improper in their Reply Brief (now designated as a Response to Non-Final Office Action). Applicants maintain that this rejection is improper for those reasons and request withdrawal of this rejection.

(2) In support of the assertion that there is "a poor correlation between gene amplification and mRNA expression," the Examiner cites Pennica *et al.*, Konopka *et al.* (Examiner's Answer pages 5, 11, 14, and 18)

Applicants disagree for the reasons set forth in their Reply Brief (now designated as a Response to Non-final Office Action). The test is whether it is more likely than not that gene amplification results in overexpression of the mRNA of the gene. In order to meet that standard, the Examiner must provide evidence that it is more likely than not that gene amplification does not result in overexpression. Accordingly, Applicants maintain that the Examiner has not met the burden.

On the other hand, Applicants have submitted ample evidence to show that, in general, if a gene is amplified in cancer, it is more likely than not that the encoded protein will be expressed at an elevated level. The articles by Orntoft *et al.*, Hyman *et al.*, and Pollack *et al.*, (made of record in Applicants' Response filed November 3, 2004) collectively teach that in general, gene amplification increases mRNA expression. Accordingly, this rejection is improper.

(3) In support of the assertion that even if increased mRNA levels could be established for PRO269, it does not follow that polypeptide levels would also be amplified, the Examiner cites Chen *et al*, LaBaer, Hu *et al.*, Haynes *et al.*, Gygi *et al.*, Lian et al., Fessler *et al.*, and Greenbaum *et al.* (Examiner's Answer, pages 6 – 8, 10, 11, 14 - 17). The Examiner divides these references into two groups: evidence that mRNA does not correlate with increased polypeptide levels in healthy tissue (Haynes *et al.*, Gygi *et al.*, Lian *et al.* and Fessler *et al.*) or cancerous tissue (see Hu *et al.*, LaBaer, Chen *et al.*, and Hanna *et al.*) (Examiner's Answer page 29)

The Examiner repeatedly offers Hu *et al.* as allegedly analyzing 2286 genes that showed a greater than 1-fold difference in mean expression level between breast cancer samples and

normal samples in a microarray. Hu *et al.* allegedly discovered that for genes displaying a 5-fold change or less in tumors compared to normal, there was no evidence of a correlation between altered gene expression and a known role in the disease. However, among genes with a 10-fold or more change in expression level, there was allegedly a strong and significant correlation between expression level and a published role in the disease. (Examiner's Answer, pages 6, 15, 20, 27 and 35-36) The Examiner states that based on Hu *et al.* the skilled artisan allegedly would not reasonably expect PRO269 protein can be used as a cancer diagnostic. (Examiner's Answer page 31)

In their Appeal Brief, Applicants discussed the reasons why Hu *et al.* did not establish a *prima facie* case for lack of utility. The Hu *et al.* reference entitled "Analysis of Genomic and Proteomic Data using Advanced Literature Mining" (emphasis added), drew conclusions based upon statistical analysis of information obtained from published literature, and not from experimental data. The statistical analysis by Hu *et al.*, is not a reliable standard because the frequency of citation only reflect the current research interest of a molecule but not the true biological function of the molecule. It often happens that important molecules are overlooked and not published. Therefore, Applicants submit that Hu *et al.* drew their conclusions based on information which is limited. Appellant also criticizes Hu *et al.* as being limited to a specific type of breast tumor (estrogen receptor positive breast tumor). Accordingly, Hu *et al.* is not sufficient evidence to show that it is likely that PRO269 protein is not overexpressed. The Examiner does not present any meaningful arguments why these criticisms wrong. The Examiner indicates that Appellant is holding Hu to a higher standard than their own specification for statistical analysis. However, Applicants have compared the level of amplification of the PRO269 gene in normal and lung tumors and have provided information indicating a greater than 2 fold amplification. Applicants are not relying on statistical analysis of information obtained from published literature based on the current research interest of a molecule. Applicants are not holding Hu et al to a higher standard. Accordingly, Hu et al. is irrelevant to the instant discussion.

The Examiner repeatedly states that Dr. LaBaer allegedly made an even stronger statement that reports of mRNA or protein changes of as little as two-fold are not uncommon, and although changes of this magnitude may turn out to be important, most are attributable to

disease-independent differences between the samples. (Examiner's Answer, pages 6, 15, 20, 36).

Similarly, the comments by LaBaer *et al.* entitled "Mining the literature and large datasets" is also based on statistical analysis like Hu, and offers an automated literature mining tool termed MedGene to comprehensively summarize gene-disease relationships. As was argued in the Hu reference, "some molecules may have been underrepresented merely because they were less frequently cited or studied in literature compared to other more well-cited or studied genes." Statistical analysis using literature mining is a very useful tool to assist the researcher in their analysis but may greatly over represent or under represent certain genes and thus their conclusions may not be generally applicable. Accordingly, the statements by LaBaer are misplaced here.

The Examiner Chen et al. as allegedly comparing mRNA and protein expression for a cohort of genes in the same lung carcinomas. Only 17% of 165 protein spots or 21% of the genes had a significant correlation between protein and mRNA expression levels. Chen et al., allegedly clearly state that "the use of mRNA expression patterns by themselves, however, is insufficient for understanding the expression of protein products' (p. 304) and "it is not possible to predict overall protein expression levels based on average mRNA abundance in lung cancer samples" (pp.311-312). (Examiner's Answer page 6, 14-15, 20, 27, 31, and 35)

The manner in which the Chen data was averaged and analyzed is a vastly different manner from that of the instant specification. For example, Chen *et al.* studied expression levels across a set of samples which included a large number of tumor samples (76) and a much smaller group of normal samples (9). The authors determined the global relationship between mRNA and corresponding protein expression using the average expression values for all 85 lung tissue samples. The authors chose an arbitrary threshold of 0.115 for the correlation to be considered significant. This resulted in negative normalized protein values in some cases and the authors concluded that it is not possible *to predict* overall protein expression based on **average mRNA abundance**. Once again, Applicants remind the Examiner that the utility standard does not require accurate prediction of protein values; only that in a majority of the proteins studied, it is more likely than not that protein levels increased when mRNA levels increased. A review of the correlation coefficient data presented in the Chen *et al.* paper indicates that, in fact, Chen teaches that 'it is more likely than not' that increased mRNA expression correlates well with increased

protein expression. For instance, a review of Table 1, which lists 66 genes [the paper incorrectly states there are 69 genes listed] for which only one protein isoform is expressed, shows that 40 genes out of 66 had a positive correlation between mRNA expression and protein expression. This clearly meets the test of "more likely than not". Similarly, in Table II , 30 genes with multiple isoforms [again the paper incorrectly states there are 29] were presented. In this case, for 22 genes out of 30, at least one isoform showed a positive correlation between mRNA expression and protein expression. Furthermore, 12 genes out of 29 showed a strong positive correlation [as determined by the authors] for at least one isoform. **No genes showed a significant negative correlation**. It is not surprising that not all isoforms are positively correlated with mRNA expression. Thus, Table II also provides that it is more likely than not that protein levels will correlate with mRNA expression levels.

The same authors in Chen *et al.*, published a later paper, Beer *et al.*, Nature Medicine 8(8) 816-824 (2002) (copy enclosed) which described gene expression of genes in adenocarcinomas and compared that to protein expression. In this paper they report that " these results suggest that the oligonucleotide microarrays provided reliable measures of gene expression" (page 817). The authors also state, "these studies indicate that many of the genes identified using gene expression profiles are likely relevant to lung adenocarcinoma". Clearly the authors of the Chen paper agree that microarrays provide a reliable measure of the expression levels of the gene and can be used to identify genes whose overexpression is associated with tumors.

The Examiner cites Hanna and Mornin as showing that gene amplification does not reliably correlate with polypeptide over-expression and thus the level of polypeptide expression must be tested empirically (Examiner's Answer page 31)).

Applicants disagree. Hanna and Mornin describe HER-2/neu Breast cancer predictive testing methods which have been FDA approved: immunohistochemistry and fluorescent in situ hybridization. While Hanna and Mornin indicate that some subsets of tumors were found lacking protein overexpression with gene amplification, Hanna and Mornin state that "in general, FISH and IHC results correlate well." (Column 2) Accordingly, it is more likely than not that protein expression with correlate with gene amplification.

The Examiner has cited Haynes *et al.*, Gygi *et al.*, Lian *et al.*, Fessler *et al.*, and Greenbaum *et al.*, as allegedly showing that increased mRNA levels do not correlate with

increased protein levels in healthy tissues.

Initially, Applicants note that these analyses were done in healthy tissues and are not a comparison of an increase in mRNA expression in cancer tissues compared to healthy tissues. Accordingly, they are not relevant to the current application.

The Examiner has cited Haynes *et al.* as providing evidence that there is "**no strong correlation** between polypeptide and transcript level. For some genes, equivalent mRNA levels translated into polypeptide abundances which varied more than 50-fold. Haynes et al. concluded that the polypeptide levels cannot be accurately predicted from the level of the corresponding mRNA transcript. (Examiner's Answer page 6-7, 15)

Applicants submit that it is not a legal requirement to establish a necessary or "strong" correlation between an increase in the copy number of the mRNA and protein expression levels that would correlate to the disease state, nor is it imperative to find evidence that DNA amplifications are always associated with overexpression of the gene product. As discussed above, the evidentiary standard to be used throughout *ex parte* examination of a patent application is a preponderance of the totality of the evidence under consideration. Accordingly, the question is whether it is more likely than not that a person of ordinary skill in the art would recognize a positive correlation.

Contrary to the Examiner's reading, Haynes *et al.* teaches that "there was a *general trend but no strong correlation* between protein [expression] and transcript levels" (Emphasis added). For example, in Figure 1, there is a positive correlation between mRNA and protein levels amongst most of the 80 yeast proteins studied. In fact, very few data points deviated or scattered away from the expected normal and no data points showed a negative correlation between mRNA and protein levels (i.e. an increase in mRNA resulted in a decrease in protein levels). Applicants further note that Haynes *et al.* was studying yeast cells and not human cells. Haynes *et al.* notes that their analysis focused on the 80 most abundant proteins in the yeast lysate (page 1867). Haynes *et al.* states "since many important regulatory proteins are present only at low abundance, these would not be amenable to analysis" (page 1867). Further, Haynes *et al.* compared the protein expression levels of these naturally abundant proteins to mRNA expression levels from published SAGE frequency tables. (page 1863). Accordingly, Haynes *et al.* did not compare mRNA expression levels and protein levels in the same yeast cells. Thus the analysis

by Haynes *et al.* is not applicable to the present application.

The Examiner argues that Applicants criticism of Haynes that it is pertinent only to yeast cells is misplaced. The Examiner refers to the references of Lian et al., Fessler et al., Hu et al., LaBaer, Chen et al., Hanna et al., and Greenbaum et al. as showing the same trend in other mammalian systems. (Examiner's Answer, page 19) Applicants disagree that these references show that mRNA levels do not predict protein levels for the reasons set forth herein. Applicants note that Greenbaum also studied yeast cells.

The Examiner Gygi *et al.* as allegedly concluding "the correlation between mRNA and protein levels was insufficient to predict protein expression levels from quantitative mRNA data. Indeed, for some genes, while the mRNA levels were of the same value, the protein levels varied by more than 20-fold.... Our results clearly delineate the technical boundaries of current approaches for quantitative analysis of protein expression and reveal that simple deduction from mRNA transcript analysis is insufficient." (Examiner's Answer page 7, 15-16, and 36)

Applicants further submit that Gygi *et al* too did not indicate that a correlation between mRNA and protein levels does not exist. Gygi *et al.* only state that the correlation may not be sufficient in **accurately** predicting protein level from the level of the corresponding mRNA transcript (Emphasis added) (see page 1270, Abstract). *Accurate prediction* is not a criteria that is necessary for meeting the utility standards. In fact, contrary to the Examiner's statement, the Gygi data also indicates **a general trend** of correlation between protein [expression] and transcript levels (Emphasis added). For example, as shown in Figure 5, the mRNA abundance of **250-300** copies /cell correlates with the protein abundance of **500-1000** x 10^3 copies/cell. The mRNA abundance of **100-200** copies/cell correlates with the protein abundance of **250-500** x 10^3 copies/cell (emphasis added). Therefore, high levels of mRNA **generally** correlate with higher levels of proteins. In fact, most data points in Figure 5 did not deviate or scatter away from the general trend of correlation. Furthermore, Gygi *et al.* studied yeast cells and not the difference in expression between normal human and lung tumor cells. *Thus*, the Gygi data, meets the "more likely than not standard" and shows that a positive correlation exists between mRNA and protein. Therefore, Applicants submit that the Examiner's rejection is based on a misrepresentation of the scientific data presented in Gygi *et al.*

Applicants submit Futcher (copy enclosed) in response to the citation of Gygi. Futcher *et*

al. (Mol. Cell. Biol. 19:7357-7368 (1999)) analyzed the yeast proteome using 2D gel electrophoresis, gathering quantitative data on protein abundance for about 1,400 spots. This data was compared to mRNA abundance for each gene as determined both by SAGE (serial analysis of gene expression) and by hybridization of cRNA to oligonucleotide arrays. The authors concluded that "**several statistical methods show a strong and significant correlation between mRNA abundance and protein abundance**" (page 7360, col. 2; emphasis added).

The authors note that Gygi *et al.* completed a similar study that generated broadly similar data, but reached different conclusions. Futcher *et al.* note that this is in part a difference in viewpoint, in that "Gygi *et al.* focus on the fact that the correlations of mRNA and codon bias with protein abundance are far from perfect" (page 7367, col. 1). Applicants respectfully submit that a showing that mRNA levels can be used to "accurately predict" the precise levels of protein expression is not required. Applicants need only show that there is a correlation between mRNA and protein levels, such that mRNA overexpression generally predicts protein overexpression. The data of both Futcher *et al.* and Gygi *et al.* clearly meets this standard.

Futcher *et al.* also point out that "the different conclusions are also partly due to different methods of statistical analysis, and to real differences in data." Futcher *et al.* first note that Gygi *et al.* used the Pearson product-moment correlation coefficient (r_p) to measure the covariance of mRNA and protein abundance. Futcher *et al.* point out that "the r_p correlation is a parametric statistic and so requires variates following a bivariate normal distribution; that is, it would be valid **only if both mRNA and protein abundances were normally distributed**" (page 7367, col. 1; emphasis added). As the authors disclose, "both distributions are very far from normal," and thus "a calculation of r_p is inappropriate" (page 7367, col. 1).

In contrast, Futcher *et al.* used two different statistical approaches to determining the correlation between mRNA and protein abundances. First, they used the Spearman rank correlation coefficient (r_s), an nonparametric statistic that does not require the data to be normally distributed. Using the r_s, the authors found that mRNA abundance was well correlated with protein abundance ($r_s = 0.74$). Applying this statistical approach to the data of Gygi *et al.* **also** resulted in a good correlation ($r_s = 0.59$), although the correlation was not quite as strong as for the Futcher *et al.* data. In a second approach, Futcher *et al.* transformed the mRNA and protein data to forms where they were normally distributed, in order to allow calculation of an r_p. Two

types of transformation (Box-Cox and logarithmic) were used, and **both** resulted in good correlations between mRNA and protein abundance for Futcher *et al.*'s data.

Futcher *et al.* also note that the two studies used different methods of measuring protein abundance. Gygi *et al.* cut spots out of each gel and measured the radiation in each spot by scintillation counting, whereas Futcher *et al.* used phosphorimaging of intact gels coupled to image analysis. Futcher *et al.* point out that Gygi *et al.* may have systematically overestimated the amount of the lowest-abundance proteins, because of the difficulty in accurately cutting out very small spots from the gel, and because of difficulties in background subtraction for small, weak spots. In addition, Futcher *et al.* note that they used both SAGE data and RNA hybridization data to determine mRNA abundances, which is most helpful to accurately measure the least abundant mRNAs. As a result, while the Futcher data set "maintains a good correlation between mRNA and protein abundance even at low protein abundance" (page 7367, col. 2), the Gygi data shows a strong correlation for the most abundant proteins, but a poor correlation for the least abundant proteins in their data set. Futcher *et al.* conclude that "the poor correlation of protein to mRNA for the nonabundant proteins of Gygi *et al.* may reflect difficulty in accurately measuring these nonabundant proteins and mRNAs, rather than indicating a truly poor correlation in vivo" (page 7367, col. 2).

Accordingly, the results of Futcher *et al.* demonstrate "a strong and significant correlation between mRNA abundance and protein abundance" (page 7360, col. 2). Further, Futcher *et al.* show that when corrected for an inappropriate statistical analysis and systematic error in the measurement of low abundance proteins, the data of Gygi *et al.* **also** meets the "more likely than not standard" and shows that a positive correlation exists between mRNA levels and protein levels.

The Examiner Lian *et al.* as allegedly showing a similar lack of correlation in mammalian (mouse) cells. "The results suggest a poor correlation between mRNA expression and protein abundance, indicating that it may be difficult to extrapolate directly from individual mRNA changes to corresponding ones in protein levels". (Examiner's Answer page 7, 16, 21, and 36)

Regarding Lian *et al.*, Applicants submit that they only teach that protein expression may not correlate with mRNA level in <u>differentiating myeloid cells</u> and does not teach anything regarding such a lack of correlation for genes <u>in general</u>. In addition, the authors themselves

admit that there are a number of problems with the data presented in this reference. At page 520 of this article, the authors explicitly express their concerns by stating that "[t]hese data must be considered with several caveats: membrane and other hydrophobic proteins and very basic proteins are not well displayed by the standard 2DE approach, and **proteins presented at low level will be missed**. In addition, to simplify MS analysis, we used a Coomassie dye stain rather than silver to visualize proteins, and this **decreased the sensitivity of detection of minor proteins.**" (Emphasis added). It is known in the art that Coomassie dye stain is a very insensitive method of measuring protein. This suggests that the authors relied on a very insensitive measurement of the proteins studied. The conclusions based on such measurements can hardly be accurate or generally applicable.

The Examiner also asserts that Fessler *et al.*, who examined lipopolysaccharide-activated neutrophilins, "found a 'poor concordance between mRNA transcript and protein expression changes' in human cells." (Examiner's answer page 7, 16, and 37).

Again, as with Lian *et al.*, Fessler *et al.* only examined the expression level of **a few proteins/RNAs** in response to LPS stimulation. Additionally, the PTO has overlooked a number of limitations of the study by Fessler *et al.* For example, as admitted by Fessler *et al.*, protein identification by two-dimensional PAGE is limited to well-resolved regions of the gel, may perform less well with hydrophobic and high molecular weight proteins, and tends to select for more abundant protein species (page 31301, col. 1). Harvesting of the LPS-incubated PMNs at 4 hours may have prevented detection of earlier, **transient changes and may have thereby introduced artificial transcript-protein discordance**. Furthermore, the post-LPS incubation, pre-two-dimensional PAGE cell washes **would be expected to remove secreted proteins from further analysis**. In addition, because protein binding of Coomassie Blue has a limited dynamic range and is typically not linear throughout the range of detection, image analysis of Coomassie Blue-stained protein spots should only be consider as semi-quantitative (see page 31301, col. 1). Again, in this study, low abundance proteins were underrepresented. Therefore, Fessler's study cannot be applied to the present application.

In summary, both Fessler *et al.* and Lian *et al.* have relied on insensitive and inaccurate methods of measuring protein expression levels. The teachings of these two references cannot be relied upon to establish a *prima facie* showing of lack of utility.

The Examiner has cited Greenbaum *et al.* as:

> "caution[ing] against assuming that mRNA levels are generally correlative of protein levels. The reference teaches (p-age 117.3 2nd column) that primarily because of a limited ability to measure protein abundances, researchers have tried to find correlations between mRNA and the limited protein expression data, in the hope that they could determine protein abundance levels from the more copious and technically easier mRNA experiments. To date, however, there have been only a handful of efforts to find correlations between mRNA and protein expression levels, most notably in human cancers and yeast cells. And, for the most part, they have reported only minimal and/or limited correlations. The reference further teaches (page 117.4, 2nd column) that there are presumably at least three reasons for the poor correlations generally reported in the literature between the level of mRNA and the level pf protein, and these may be mutually exclusive. First, there are many complicated and varied post-transcriptional mechanisms involved in turning mRNA into protein that are not yet sufficiently well defined to be able to compute protein concentrations from mRNA; second, proteins may differ substantially in their *in vivo* half lives; and/or third, there is a significant amount of error and noise in both protein and mRNA experiments that limit our ability to get a clear picture. The reverence further notes (page 117.6, page 2nd column) that to be fully able to understand the relationship between mRNA and protein abundances, the dynamic processes involved in protein synthesis and degradation have to be better understood." (Examiner's Answer, pages 7 – 8, 16-17, and 37-38).

Applicants note that Greenbaum is also comparing the expression of a number of different mRNAs and their corresponding proteins in yeast cells and not comparing the change of expression of specific mRNAs and their corresponding proteins in cancer cells versus normal cells. Accordingly, this reference is also not relevant to the issue at hand. Nevertheless, Greenbaum states that logically "we would assume that those ORFs that show a large degree of variation in their expression are controlled at the transcriptional level. The variability of the mRNA expression is indicative of the cell controlling the mRNA expression at different points of the cell cycle to achieve the resulting and desired protein. **Thus we would expect and we found a high degree of correlation (r-0.89) between the reference mRNA and protein levels for these particular ORFs: the cell has already put significant energy into dictating the final level of protein through tightly controlling the mRNA expression**. (page 117.5 1st column). Furthermore, Greenbaum states : **"we found that ORFs that have higher than average levels of ribosomal occupancy – that is that a large percentage of their cellular mRNA concentration is associated with ribosomes (being translated) – have well correlated mRNA and protein expression levels. (Figure 2)."** Therefore, contrary to the Examiner's assertion, Greenbaum does find high levels of correlation between mRNA and protein expression in yeast cells.

For the reasons given above, Applicants respectfully submit that the Examiner has not established a *prima facie* showing of lack of utility based on the new references cited in the Examiner's answer either and therefore, the Patent Office has failed to meet its initial burden of proof.

Applicants submit herewith further evidence that increased mRNA expression in cancer as compared to normal tissues is correlated with increased protein expression in the same cancerous tissues as compared to normal tissues.

Maruyama *et al.* (Am. J. Pathol. 155:815-822 (1999); copy enclosed) investigated the expression of three Id proteins (Id-1, Id-2 and Id-3) in normal pancreas, in pancreatic cancer and in chronic pancreatitis (CP). The authors report that all three Id mRNA species were expressed at high levels in pancreatic cancer cells as compared to normal or CP samples, and that the pancreatic cancer cell lines also exhibited "a good correlation between Id mRNA and protein levels" (Abstract). The authors measured both mRNA and protein expression in five different human pancreatic cancer cell lines. The authors observed a correlation between mRNA and protein expression of Id1 in all five cell lines, and a correlation between mRNA and protein expression for Id2 and Id3 in four out of five cell lines. In these discordant cases, Id protein levels were increased while mRNA levels were not. As noted above, Applicants make no assertions regarding changes in protein levels when mRNA levels are unchanged, nor does evidence of changes in protein levels when mRNA levels are unchanged have any relevance to Appellants' asserted utility. Thus, the authors report that increased mRNA levels leads to an increase in protein overexpression, supporting Applicants' assertion.

Bea *et al.* (Cancer Res. 61:2409-2412 (2001); copy enclosed) investigated gene amplification, mRNA expression, and protein expression of the putative oncogene BMI-1 in lymphoma samples. The authors examined BMI-1 protein expression in 31 tumors for which levels of gene amplification and mRNA expression had been determined. Bea *et al.* found that "[a] good correlation between BMI-1 mRNA levels and protein expression was observed in all types of lymphomas" (Abstract). Thus, the authors report that increased mRNA levels leads to an increase in protein overexpression, supporting Applicants' assertion.

Papotti *et al.* (Diagn Mol Pathol. 9(1):47-57 (2000); copy enclosed) studied the somatostatin type 2 receptor (sst2) in 26 different neuroendocrine lung tumors. They

investigated mRNA levels by RT-PCR and protein levels by immunohistochemistry using 2 different antibodies. The authors report that "in the majority of samples a good correlation between sst2 mRNA (as detected by RT-PCR) and sst2 protein expression (as detected by immunohistochemistry) was observed" (Abstract). The authors also performed *in situ* hybridization (ISH) in selected samples which "paralled the results obtained with the other techniques" (Abstract).

Walmer *et al.* (Cancer Res. 55(5):1168-75 (1995); copy enclosed) looked at lactoferrin mRNA and protein expression in endometrial adenocarcinomas and report that two thirds (8 of 12) of the samples examined overexpress lactoferrin. Walmer *et al.* also found that "this tumor-associated increase in lactoferrin expression includes an elevation in the mRNA and protein of individual cells" and that "serial sections of malignant specimens show(ed) a good correlation between the localization of lactoferrin mRNA and protein in individual epithelial cells by *in situ* RNA hybridization and immunohistochemistry" (Abstract).

Janssens *et al.* (Tumour Biol. 25(4):161-71 (2004); copy enclosed) evaluated the involvement of frizzled receptors (Fzds) in oncogenesis. They investigated mRNA expression levels in 30 different human tumor samples and their corresponding (matched) normal tissue samples by real-time quantitative PCR. Janssens *et al.* observed markedly increased Fzd5 mRNA levels in 8 of 11 renal carcinoma samples and that "Western blot analysis of crude membrane fractions revealed that Fzd5 protein expression in the matched tumor/ normal kidney samples correlated with the observed mRNA level" (Abstract).

Hahnel *et al.* (Breast Cancer Res Treat. 24(1):71-4 (1992); copy enclosed) studied expression of the pS2 gene in breast tissues by measuring mRNA levels using Northern blotting and protein levels by radioimmunoassay. Hahnel *et al.* indicate that "there was a good correlation between the two measurements, indicating that expression of the pS2 gene in breast tissues may be assessed by either method".

Kammori *et al.* (Int J Oncol.. 27:1257-63 (2005); copy enclosed) studied the expression of human telomerase reverse transcriptase (hTERT) gene and protein (besides estrogen and progesterone receptors) in breast tumors using *in situ* hybridization (ISH) for mRNA and immunohistochemistry (IHC) for the protein. They looked at 64 adenocarcinomas, 2 phyllode tumors and their adjacent normal breast tissues and found that hTERT mRNA was detected in 56

tumors but in neither of the 2 phyllode tumors whereas hTERT protein expression was detected by IHC in 52 tumors but in neither of the 2 phyllode tumors. The authors concluded that "there was a strong correlation between detection of hTERT gene expression by ISH and of hTERT protein by ICH in tissue specimens from breast tumors" (Abstract).

With regard to the correlation between mRNA expression and protein levels, Applicants submitted a first Declaration by Dr. Polakis (Polakis I) , principal investigator of the Tumor Antigen Project of Genentech, Inc., the assignee of the present application, to show that mRNA expression correlates well with protein levels, in general. As Dr. Polakis explains, the primary focus of the microarray project was to identify tumor cell markers useful as targets for both the diagnosis and treatment of cancer in humans.

While the proper legal standard is to show that the existence of correlation between mRNA and polypeptide levels is more likely than not, the showing of approximately 80% correlation for the molecules tested in the Polakis Declaration greatly exceed this legal standard. Based on these experimental data and his vast scientific experience of more than 20 years, Dr. Polakis states that, for human genes, increased mRNA levels typically correlate with an increase in abundance of the encoded protein. He further confirms that "it remains a central dogma in molecular biology that increased mRNA levels are predictive of corresponding increased levels of the encoded protein."

The Examiner has stated that Dr. Polakis' Declaration is allegedly not persuasive because the specification only provides information regarding PRO269 gene amplification data and does not disclose PRO269 mRNA data. Furthermore, there is allegedly strong opposing evidence showing that gene amplification is not predictive of increased mRNA levels in normal and cancerous tissues and in turn that increased mRNA levels are not predictive of increased polypeptide levels. (Examiner's Answer, page 26 – 27).

The Examiner states that while Dr. Polakis bases his findings on facts, the facts are allegedly not independently provided for the examiner to draw independent conclusions. For example it allegedly is not clear if any of the tumors were from lung or how highly amplified the genes were that correlated with polypeptide expression.

Without acquiescing to the propriety of this rejection, merely to expedite prosecution in this case, Applicants present a second Declaration by Dr. Polakis (Polakis II) that presents

evidentiary data in Exhibit B. Exhibit B of the Declaration identifies 28 gene transcripts out of 31 gene transcripts (i.e., greater than 90%) that showed good correlation between tumor mRNA and tumor protein levels. As Dr. Polakis' Declaration (Polakis II) says "[a]s such, in the cases where we have been able to quantitatively measure both (i) mRNA and (ii) protein levels in both (i) tumor tissue and (ii) normal tissue, we have observed that in the vast majority of cases, there is a very strong correlation between increases in mRNA expression and increases in the level of protein encoded by that mRNA. Accordingly, Dr. Polakis has provided the facts to enable the Examiner to draw independent conclusions.

The case law has clearly established that in considering affidavit evidence, the Examiner must consider all of the evidence of record anew.[1] "After evidence or argument is submitted by the applicant in response, patentability is determined on the totality of the record, by a preponderance of the evidence with due consideration to persuasiveness of argument"[2] Furthermore, the Federal Court of Appeals held in *In re Alton*, "We are aware of no reason why opinion evidence relating to a fact issue should not be considered by an examiner"[3]. Applicants also respectfully draw the Examiner's attention to the Utility Examination Guidelines[4] which state, "Office personnel must accept an opinion from a qualified expert that is based upon relevant facts whose accuracy is not being questioned; it is improper to disregard the opinion solely because of a disagreement over the significance or meaning of the facts offered."

Taken together, although there are some examples in the scientific art that do not fit within the central dogma of molecular biology that there is a correlation between polypeptide and mRNA levels, these instances are exceptions rather than the rule. In the majority of amplified genes, the teachings in the art, as exemplified by Orntoft *et al.*, Hyman *et al.*, Pollack *et al.*, Bea et al., Maruyama et al., Futcher et al., Papotti *et al.*, Walmer *et al.*, Janssens *et al.*, Hahnel *et al. and* Kammori *et al.* and the Polakis Declarations, overwhelmingly show that gene amplification

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[1] *In re Rinehart,* 531 F.2d 1084, 189 USPQ 143 (C.C.P.A. 1976) and *In re Piasecki,* 745 F.2d. 1015, 226 USPQ 881 (Fed. Cir. 1985).

[2] *In re Alton,* 37 USPQ2d 1578 (Fed. Cir 1966) at 1584 quoting *In re Oetiker,* 977 F.2d 1443, 1445, 24 USPQ2d 1443, 1444 (Fed. Cir. 1992)).

[3] *In re Alton, supra.*

[4] Part IIB, 66 Fed. Reg. 1098 (2001).

influences gene expression at the mRNA and protein levels. Therefore, one of skill in the art would reasonably expect in this instance, based on the amplification data for the PRO269 gene, that the PRO269 polypeptide is concomitantly overexpressed. Thus, Applicants submit that the PRO269 polypeptides and antibodies have utility in the diagnosis of cancer and based on such a utility, one of skill in the art would know exactly how to use the antibody for diagnosis of cancer.

Accordingly, this rejection under 35 U.S.C. §101 and §112, first paragraph, should be withdrawn.

CONCLUSION

For the reasons given above, Applicants submit that the gene amplification assay disclosed in Example 92 of the specification, and the advanced state of the art in oncology, provide at least one patentable utility for the PRO269 polypeptides of Claims 39-43, and that one of ordinary skill in the art would understand how to use the claimed polypeptides and would have found such testing routine and not 'undue.' Therefore, Claims 39-43 meet the requirements of 35 U.S.C. §101 and 35 U.S.C. §112, first paragraph.

Please charge any additional fees, including any fees for additional extension of time, or credit overpayment to Deposit Account No. **08-1641** (referencing Attorney's Docket No. **39780-1618 P2C34**).

Respectfully submitted,

Date: March 30, 2006

By: _____
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